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A study on the effects of cottonseed cake on histopathological changes of liver in sheep

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Abstract
Histopathological changes in liver of six male sheep were studied by feeding different levels (40%, 25% and 0%) of cottonseed cake in the total diets for two months. Liver of sheep from groups A and B revealed degeneration and necrosis of hepatocytes, degeneration of central vein and wider sinusoidal spaces. In the portal area, degeneration of branch of portal vein, hepatic arteriole and branch of bile duct, connective tissue infiltration and oedematous fluid in portal area were found. Congestion in the branch of portal vein and central vein was observed. Histopathological changes in liver of group A were more severe than that of group B. Feeding of 40% cottonseed cake in the diet caused more toxic effects to sheep than that of 25% cottonseed cake in the diet.

Keywords: Cottonseed cake, gossypol, histopathological changes, liver, sheep

1. Introduction
Cottonseed cake, being rich in protein content, is used extensively for supplementing in the ruminant ration to increase productivity (Zahid *et al*., 2002) [18]. However, usage of cottonseed cake as animal feed for both ruminants and non-ruminants is restricted because of high levels of toxic substance, gossypol in cottonseed and cottonseed products (Matondi *et al*., 2007) [7].
Gossypol is a naturally occurring lipid soluble polyphenolic compound present in the seeds, stems and roots of cotton plant genus *Gossypium* (Price *et al*., 1993) [10]. Continuous feeding of diets containing gossypol can cause negative effects on growth performance and reproduction both in male and female (Santos *et al*., 2003) [13]. According to Randel *et al*. (1992) [11], common clinical signs of gossypol toxicosis in monogastrics, preruminants and mature ruminants are similar and include labored breathing, dyspnea, decreased weight gain, anorexia, weakness and death after several days.
In the present study, effects of feeding cottonseed cake on histopathological changes in liver of local sheep in Myanmar were studied.

2. Materials and Methods
2.1 Experimental animals and design
Six male sheep approximately 3 months of age and weighing from 13 kg to 20 kg were used in this experiment and kept in the individual cage. Primarily, all of the experimental animals were fed with rice straw and sesame cake as basal diet during the adaptation period for one month and also provided free access of drinking water. After adaptation period, sheep were randomly allocated into three groups (A, B and C) with two animals in each group. Group C was kept as control group and fed with basal diet, and groups A and B were fed 40% (21.1 mg free gossypol/kg body weight/day) and 25% (13.2 mg free gossypol/kg body weight/day) of cottonseed cake in the total diets, respectively. Diets were formulated isonitrogenously around 16% of crude protein. Concentrate contributed 40% and rice straw 60% of the feed given to sheep amounting to 3.5% of body weight. The experimental period was two months.

2.2 Microscopic examination
At the end of the experimental period, sheep from all groups; A, B and C were sacrificed by euthanization with excess dose of barbiturate via intravenous injection.

The liver was collected for tissue sections and then immediately fixed in 10% formalin. After that, tissue processing and staining were carried out. The samples were dehydrated in the series of ascending grades of alcohol followed by clearing in the changes of xylene, and then the tissues were infiltrated with different grades of melted paraffin in the automatic tissue processor. The tissues were then embedded in paraffin and finally the wax embedded specimens were sectioned at 3-4 µm thickness. The sections were floated on Luke-warm water in a floatation bath at 37 °C for stretching and then they were mounted on clean slides. Then glass slides were dried on a slide warmer at 37 °C. Tissue section slides were stained with Harris Haematoxylin and Eosin (H & E). Histopathological slides were examined under the light microscope and microphotographed with camera attached microscope.

3. Results

The observations in this study, the diets containing cottonseed cake at different inclusion levels induced varying degrees of histopathological changes in liver of sheep. Histopathological changes such as degenerated hepatocytes and degeneration of central vein were observed in both groups A and B. The sinusoidal spaces were wider than the normal and more distinct in group A (Figure 1 and 2). Degenerations in the portal area such as degeneration of branch of portal vein and hepatic arteriole, degeneration of branch of bile duct and oedematous fluid in portal area were also found (Figure 3 and 4). Moreover, liver cell cords were degenerated and necrosis characterized by disappearance of nucleus in both groups A and B (Figure 5 and 6). Connective tissue infiltration was also seen in portal area of both groups fed with cottonseed cake (Figure 7 and 8). In group B, congestion in branch of portal vein was observed (Figure 3 and 7). In group A, congestion in central vein and disappearance of degenerated hepatocytes were revealed (Figure 9).

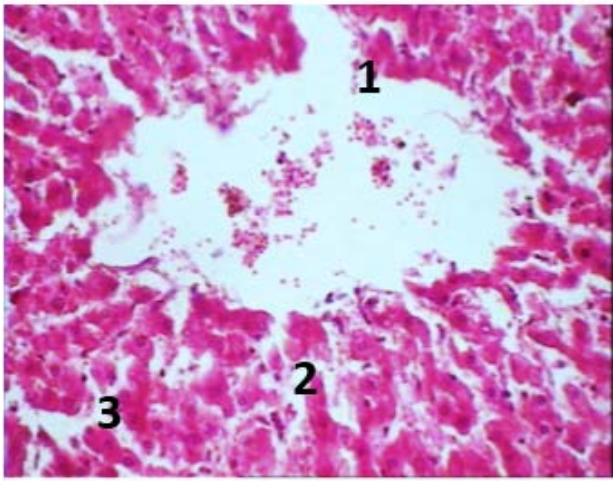

Fig 1: Microphotograph of liver of sheep in group B showing degeneration of central vein (1), degeneration of hepatocytes (2) and wider sinusoidal spaces (3) (H&E, ×400)

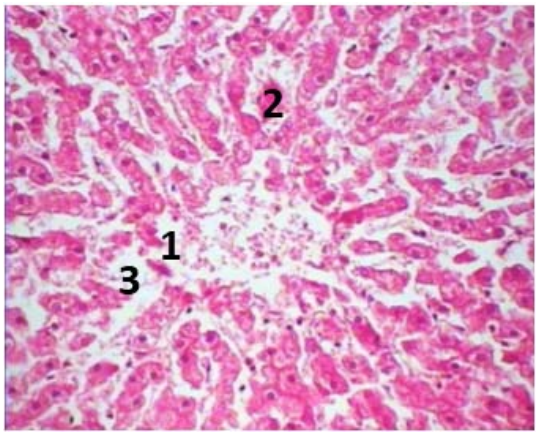

Fig 2: Microphotograph of liver of sheep in group A showing degeneration of central vein (1), degeneration and necrosis of hepatocytes (2) and wider sinusoidal spaces (3) (H&E, ×400)

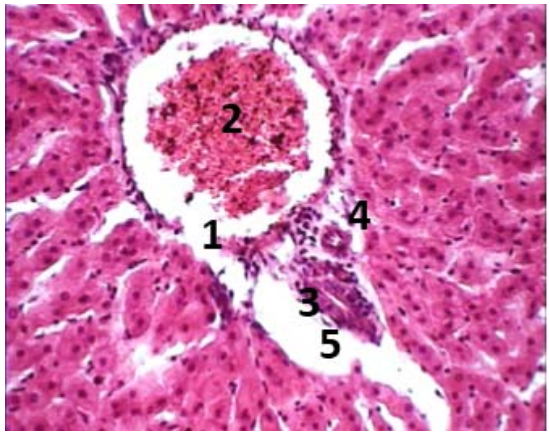

Fig 3: Microphotograph of liver of sheep in group B showing degeneration of branch of portal vein (1), congestion in branch of portal vein (2), degeneration of branch of bile duct (3) and hepatic arteriole (4) and oedematous fluid in the portal area (5) (H&E, ×400)

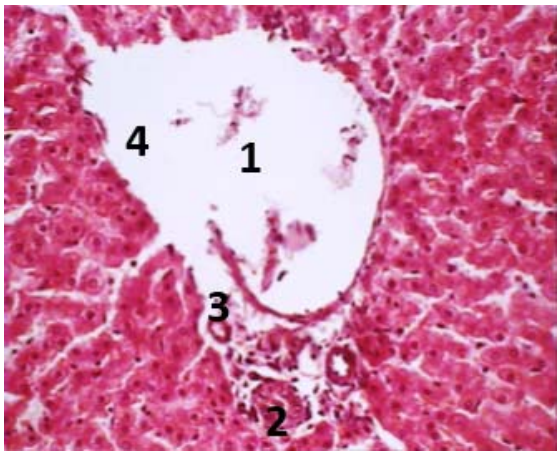

Fig 4: Microphotograph of liver of sheep in group A showing degeneration of branch of portal vein (1), branch of bile duct (2) and hepatic arteriole (3) and oedematous fluid in the portal area (4) (H&E, ×400)

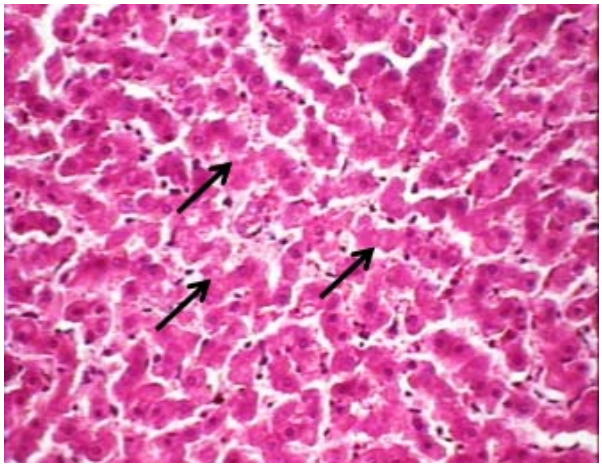

Fig 5: Microphotograph of liver of sheep in group B showing degeneration and necrosis of liver cell cords characterized by the disappearance of nucleus (arrow) (H&E, ×400)

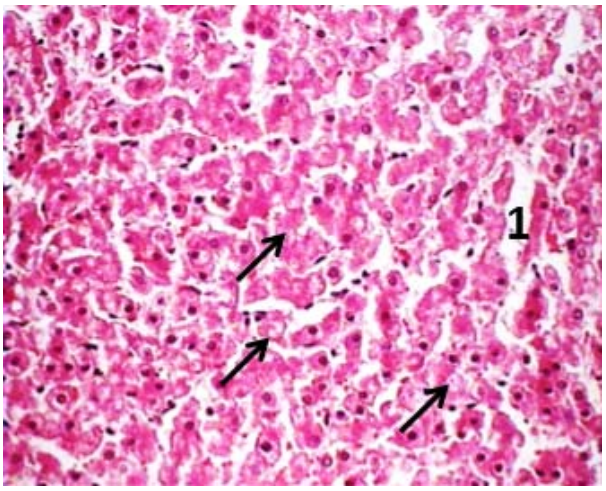

Fig 6: Microphotograph of liver of sheep in group A showing degeneration and necrosis of liver cell cords characterized by the disappearance of nucleus (arrow) and wider sinusoidal spaces (1) (H&E, × 400)

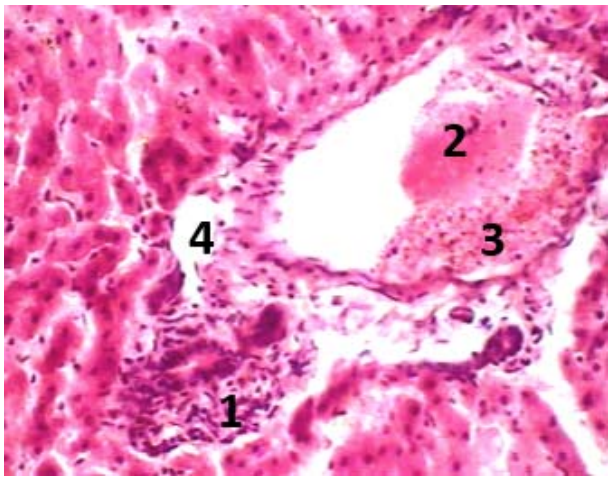

Fig 7: Microphotograph of liver of sheep in group B showing infiltration of connective tissue around the degenerated branch of bile duct (1), accumulation of proteinaceous fluid in the degenerated branch of portal vein (2), RBC in branch of portal vein (3) and oedematous fluid in portal area (4) (H&E, ×400)

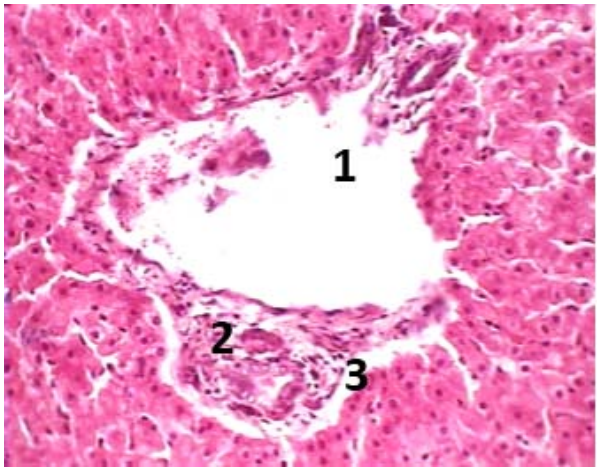

Fig 8: Microphotograph of liver of sheep in group A showing degeneration of branch of portal vein (1), infiltration of connective tissue around the degenerated branch of bile duct (2) and oedematous fluid in the portal area (3) (H&E, ×400)

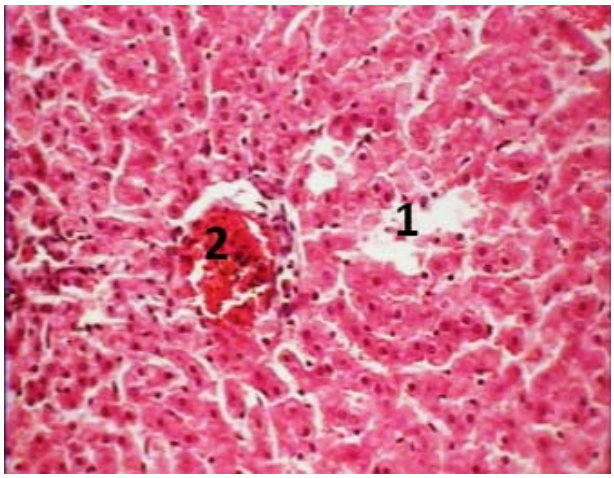

Fig 9: Microphotograph of liver of sheep in group A showing disappearance of degenerated hepatocytes (1) and congestion in central vein (2) (H&E, ×400)

4. Discussion

In the present study, liver of sheep in both groups fed with cottonseed cake showed degeneration and necrosis of hepatocytes with widening of sinusoidal spaces (Figure 1 and 2). Moreover, degeneration and necrosis of liver cell cords characterized by disappearance of nucleus was also observed in both groups A and B (Figure 5 and 6). This result was similar with the finding of El-Sharaky *et al*. (2010) [6], who mentioned that the gossypol acetic acid treated male rats had serious degenerative changes in the liver. According to Ali and El-Sewedy (1984) [1], gossypol was also hepatotoxic due to the depletion of hepatic glutathione. Glutathione was present in all types of living cells and was able to conjugate with a variety of chemically active compounds (Mitchell *et al.,* 1976) [8]. Loss of cellular glutathione was indicative of cytotoxicity (Docks and Krishna, 1976) [5]. Sufficient depletion of cellular glutathione content allowed some reactive metabolites to react with cellular macromolecules leading to cellular injury and death (Docks and Krishna, 1976; Olson *et al.,* 1980) [5, 9].

There was infiltration of connective tissues around the blood vessels at the portal area of both groups A and B (Figure 7 and 8). This was due to the destruction of the blood vessel walls and substitution of the damaged wall with the

connective tissues. Sastry (1983) [14] reported that when irritant persist for long period, the body responds by producing excessive amount of connective tissue in the injury area. Moreover, sheep from both groups A and B exhibited degeneration of branch of portal vein and hepatic arteriole and also degeneration of central vein (Figure 1, 2, 3 and 4). Furthermore, proteinaceous fluid and RBCs in branch of portal vein (Figure 7) and oedematous fluid in the portal area (3, 4, 7 and 8) were also seen. These lesions were associated with the vascular wall injury and leakage of plasma protein into tissue space (Runnells *et al*., 1965) [12]. In addition, congestion in the branch of portal vein (Figure 3) in group B and congestion in central vein (Figure 9) in group A referred to as passive hyperaemia of the liver. Passive hyperaemia of the liver is caused by hindrance to the flow of blood in the hepatic vein, posterior vena cava and heart (Runnells *et al*., 1965) [12]. According to Wu *et al*. (1991) [16], gossypol in cottonseed cake inhibited hydroxylase needed for synthesis of hydroxyproline. Szpak (2011) [15] reported that hydroxyproline is a major component of the protein collagen. The reduction in collagen content, or the more fragile character of the collagen in various organs, might induce such symptoms as vascular damage and capillary haemorrhage (Dewreede and Waymann, 1970) [4].

Furthermore, degeneration of branch of bile duct was found in Figure 3 and 4. Yanwan *et al*. (1979) [17] reported that gossypol induced distension of endoplasmic reticulum and displacement of ribosomes from rough endoplasmic reticulum of hepatic cells. In addition, Band *et al.* (1989) [2] mentioned that gossypol inhibited protein synthesis in mammalian cells. That inhibition interfered the epithelial integrity (Chernoff, 2006) [3].

5. Conclusion

From the present study, it could be concluded that the gossypol in cottonseed cake caused the degeneration and also destructions of blood vessel in liver of cottonseed cake treated groups. According to these lesions, inclusion of 40% cottonseed cake in the diet of sheep caused more toxic effect in liver than that of 25% in the diet.

6. References

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